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REPLY BRIEF Address to: Mail Stop Appeal Brief-Patents Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Application Number	10/716,349
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	Attorney Docket No.	SEEK-001CON
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	First Named Inventor	Ellen L. Berg
	Examiner	Karlheinz Skowronek
	Group Art	1631
	Title: *Function Homology Screening*	

Sir:

This Reply Brief is filed in support of Appellants' appeal from the Examiner's Answer of June 10, 2009, and is accompanied by a request for oral hearing.

The Board of Appeals and Interferences has jurisdiction over this appeal pursuant to 35 U.S.C. §134.

Appellants believe that fees are due only for the request for oral hearing. In the unlikely event that the fee transmittal or other papers are separated from this document and/or other fees or relief are required, Appellants petition for such relief, including extensions of time, and authorize the Commissioner to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, or to credit any overpayment, to deposit account number 50-0815, reference no. SEEK-001CON.

Remarks

In response to Appellants' Brief of March 2, 2009; the Examiner's Answer was mailed on June 10, 2009. The Examiner raised several points in response to Appellants' arguments, which are herein addressed.

The presently claimed invention is directed to a method of contacting a mammalian cell culture with a compound to be characterized, where the cells in the culture are activated by at least two factors. The cited reference Friend et al., in contrast, describes methods for screening molecules using cell co-cultures, wherein the cell cultures differ by expression of a single target gene by the over- or under-expression of that target gene. The Friend *et al.* methods do not employ a cell culture comprising at least two factors or in which a plurality of pathways is activated.

The Examiner has stated that it is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways as evidenced by Chung *et al.*, who demonstrate the culture of rabbit kidney cells in a culture medium. Applicants respectfully submit that it is not inherent to cell cultures to activate a plurality of pathways through multiple factors. It is possible to design a culture where a plurality of pathways is activated. Similarly it is possible to design a culture where a plurality of pathways is not activated. For example, Chung et al. found that the rabbit kidney cultures required a factor such as insulin, but cultures did not respond to factors such as EGF and T_3.

Appellants have observed that the activation of cells in multiple pathways reveals properties of test agents that are cryptic in the absence of these factors. Many biologically active agents were found to have no detectable change in parameters when brought into contact with unstimulated cells, as can be found in many cell culture systems. Yet when a biologically active agent is added to cells stimulated in multiple pathways, as in the methods of the invention, distinctive parameter changes could be observed. Accordingly, Appellants submit that "a plurality of factors that affect a plurality of signaling pathways" as set forth in the claimed invention cannot be inherently equivalent to endogenous cell-culture factors, which are asserted to be invariantly present in any cell culture system, because such endogenous factors do not inherently activate a plurality of signaling pathways.

Cells that are cultured continuously in the presence of a factor may down-regulate their receptors, such that over time they no longer respond to the factor, or become refractory. The concentration of factors may be insufficient to induce a plurality of pathways. Cells may also respond in an oscillatory fashion to factors in the culture medium, such that depending on the time point of assessment, the plurality of signaling pathways is not induced. In other cases, the presence

of one factor may abrogate the signaling activity of another, for example retinoids suppress epidermal growth factor-associated cell proliferation by inhibiting epidermal growth factor receptor-dependent ERK1/2 activation. Applicants respectfully submit that the evidence of Chung *et al.* does not inherently provide Friend *et al.* with each and every element of the present claims, which specify that a plurality of signaling pathways is induced.

In the methods of the present invention, a test agent contacts cells in culture that are stimulated in multiple pathways by the addition of at least two factors. Appellants respectfully submit that there is no teaching by Friend *et al.* as evidenced by Chung *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell.

With respect to Appellants' arguments regarding the use of a plurality of factors in cultures described by the cited art, Chung *et al.*, the examiner has stated that "It is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways as evidenced by Chung et al who demonstrate the culturing of mammalian kidney cells in a culture medium having growth promoting amounts of factors such as epidermal growth factor and insulin, among others."

Appellants submit that although the Chung reference does include a plurality of factors in the culture medium, the experimental design does not specifically address whether or not a plurality of signaling pathways are affected. In these experiments, primary cells are stimulated with combinations of insulin, transferring and hydrocortisone for 10-15 days, at which point cell numbers are assessed. Any differences in cell number that are observed at 10 and 15 days are not necessarily due to interaction of signaling pathways, but rather, could be due to selective outgrowth of cell types responding to individual factors. Indeed, the authors themselves state in the first paragraph of the discussion (p. 125, first paragraph) that the addition of different sets of factors may cause an enrichment in particular cell types in primary cultures.

This experimental set up is not compatible with the methodology of Friend et.al., from the temporal perspective, so not only is it not obvious to combine the two, but combining these two would not achieve the claims of the present invention.

Cell populations at 10-15 days that are mixtures of different subpopulations (the relative numbers of which depend on the different input conditions), would not enable the building of a reference set of agent profiles that could be used to deconvolute mechanisms of action, as we describe. The confounding effects of different combinations of subpopulation numbers would mask pathway interactions, and only reflect different numbers of subpopulation responses. In the absence of pathway interactions the effects of individual factors is simply additive, and synergistic effects would not be present to allow pathway / mechanism deconvolution.

Appellants have argued that Friend et al. fails to disclose a method in which a test agent contacts cells in culture that stimulated in multiple pathways by the addition of at least 2 factors. The Examiner asserts that Appellant's argument is not persuasive because "Friend et al. shows that to measure drug response data, cells are exposed to graded levels of the drug or drug candidates of interest (col. 34, line 42-43)".

Appellants respectfully submit that exposing cells to graded levels of drug candidate differs significantly from a method of the presently claimed invention, where a drug candidate is tested in cells that are also stimulated in multiple pathways by the addition of at least 2 factors.

Appellants have argued that the factors contemplated by the methods of the instant claims are modulatory of specific pathways in order for the data set to be informative.

In response the Examiner asserts that "the claims have been interpreted in light of the specification. The specification teaches at p. 12, paragraph [0035], line 1-3, that "assay combinations, usually employing cell cultures, are provided that simulate physiological cell states of interest, particularly physiological cell states in vivo, usually using the same type of cells or combinations of cells"". It is then stated that Chung shows a physiological culture system.

Appellants respectfully submit that the use of physiologically relevant factors is not pertinent to the point of Appellants argument, which is that the factors required to be present in the claimed cell cultures must modulate specific pathways to be informative. Indeed, the actual language of the present claims should be considered, which state in Claim 1 that:

"wherein a plurality of signaling pathways are induced by the presence of the factors . . . the measurements of the test cell culture and the control cell culture to produce a biological dataset profile, wherein the biological dataset profile is indicative of the pathways that are active in the cell culture".

Thus, the claims specifically require that a plurality of pathways be induced, such that measured parameters are indicative of the pathways that are active.

The Examiner has asserted that Appellant mischaracterizes, at p. 7, lines 16-18 of the Brief filed 2 March 2009, the teaching of Friend et al. at col. 10, line 54-59 by stating, "The cited paragraph from column 10 suggests that screening drugs for treatment of kidney cancer might use kidney cancer cells, but that in preferred embodiments, yeast cells are used".

It is asserted by the Examiner that "contrary to Appellent's characterization, Friend *et al.* states at col. 10, line 54-59, "In most preferred embodiments of the invention, the cells used for

cluster analysis are of the same type and from the same species as the species of interest. For example, human kidney cells are preferably tested to identify consensus profiles to evaluate drugs or therapies that are used to treat disorders involving human kidney cells."

Appellants submit that the Examiner has apparently omitted the rest of the cited paragraph from Friend et al, because the full citation would proceed to state that:

> However, in some preferred embodiments, the biological samples are not of the same type or are not from the same species as the species of interest. For example, in certain preferred embodiments, yeast cells may be used to define consensus profiles that are useful, e.g., in comparing or evaluating drugs or drug candidates used or intended for human therapies." (underlining added)

Appellants note that the data provided by Friend *et al.* supports the use of yeast as a preferred host organism, as the figures show only *S. cerevisiae* (yeast) transcripts (Figures 2-3); *S. cerevisiae* response to chemotherapy drugs (Figure 4-5); and then analysis of this yeast experiments in the remaining figures.

Appellants have argued that Friend *et al.* in view of Chung *et al.* fail to show the measurement of at least two parameters associated with a plurality of pathways. The Examiner asserts that "Appellants' argument is not persuasive. Friend et al. shows at col. 10, line 54-56 that "in most preferred embodiments of the invention, the cells used for cluster analysis are of the same type and from the same species as the species of interest. For example, human kidney cells are preferably tested to identify consensus profiles to evaluate drugs or therapies that are used to treat disorders involving human kidney cells"."

Appellants submit that this rebuttal is not on point. The choice of cells is a separate matter from a showing of multiple parameters associated with a plurality of pathways. Friend *et al.* generates a profile of mRNA expression, while in contrast, the present invention claims methods that utilize a step of measuring multiple cellular parameters associated with the induced signaling pathways.

Appellants have argued that the invention provides an unexpected benefit in that only when one compares a plurality of pathway components can one distinguish the action of a test agent. In rebuttal, the Examiner asserts that Appellant's argument is not persuasive because Figure 3 of Friend et al. shows a similar analysis. In figure 3, Friend et al. shows a false color display of a plurality of genetic transcripts measured in a plurality of experiments. Friend et al. shows this has the benefit that the response profiles can be readily visualized (col. 28, line 58-59).

Appellants respectfully submit that Figure 3 of Friend *et al.* does not show how one distinguishes the activation of a particular pathway by a candidate agent. Figure 3 of Friend et al. shows the transcriptional response of yeast cells to drugs. Figure 3 of the reference is as follows:

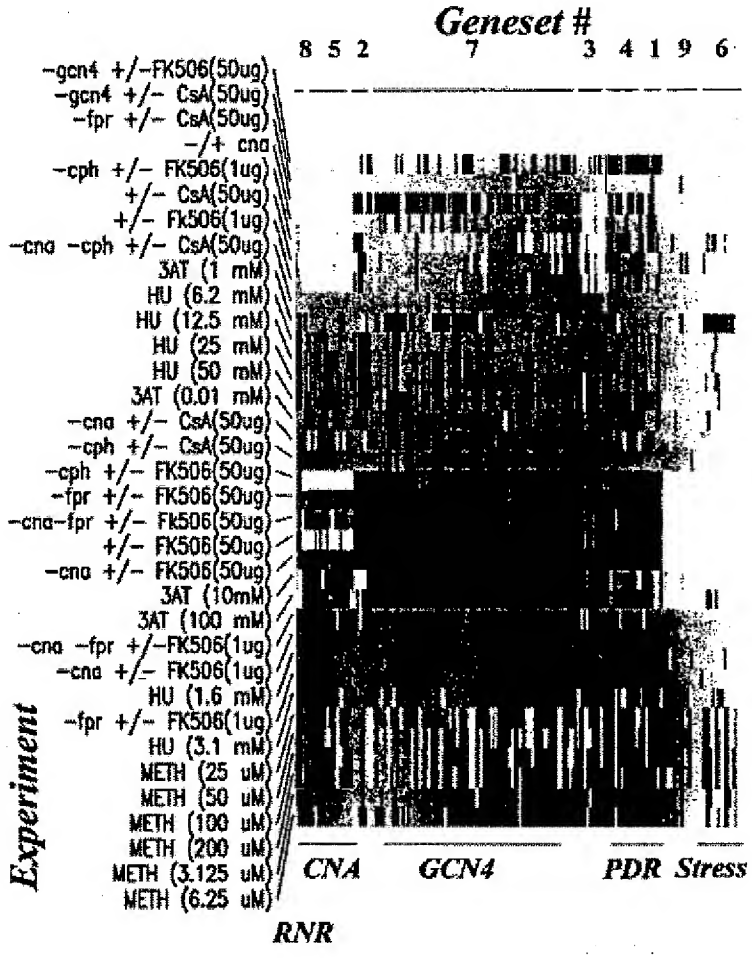

FIG.3

The Figure does not show how candidate compounds interact with signaling pathways, or show how cells grown in the presence of factors that induce a plurality of signaling pathways can function to identify the effect of a drug on those pathways. Rather, yeast cells are grown in a single medium, to which a test compound is made, and then mRNA transcripts are obtained.

The Examiner has asserted in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

Appellants submit that it is proper to address references individually when they cannot be usefully combined. As discussed above, although the Chung reference does include a plurality of factors in the culture medium, the experimental design does not specifically address whether or not a plurality of signaling pathways are affected. In these experiments, primary cells are stimulated with combinations of insulin, transferring and hydrocortisone for 10-15 days, at which point cell numbers are assessed. Any differences in cell number that are observed at 10 and 15 days are not necessarily due to interaction of signaling pathways, but rather, could be due to selective outgrowth of cell types responding to individual factors. Indeed, the authors themselves state in the first paragraph of the discussion (p. 125, first paragraph) that the addition of different sets of factors may cause an enrichment in particular cell types in primary cultures.

This experimental set up is not compatible with the methodology of Friend et al., from the temporal perspective, so not only is it not obvious to combine the two, but combining these two would not achieve the claims of the present invention.

Indeed, Appellants submit that the Examiner is attempting to reconstruct Appellants invention by selectively reviewing the literature to obtain separate aspects of Appellants invention, without providing a teaching of the unified whole that is required to obtain the unexpected benefits of Appellants work.

The present claims are drawn to various methods for generating and utilizing a dataset of parameter values obtained from cells under specific culture conditions – termed a "biomap" or a "biomap profile" – in determining the effect of an agent on a cellular signaling pathway. The subject methods provide robust results having enhanced predictability in relation to a physiological state of interest, by providing for the culture systems where multiple pathways are induced, and where multiple parameters are measured and compared to control assay combinations.

Applicants' invention provides methods that harness cellular complexity to provide insight into the pathways affected by a candidate drug of interest. Such analysis provides a much deeper understanding of gene action than can be readily obtained by methods taught by the prior art, and such a deeper understanding is very useful in drug development, elucidation of cellular signaling pathways, and the like.

Biological responses, particularly responses in primary human cells, can display significant variability from day to day and from donor to donor. One important aspect of the present invention is that, while the levels of determined parameters can vary substantially between assays, combinatorial responses involving multiple pathways are less variable. Thus, the process of normalization used to produce a biomap provides cellular activity profiles that are robust and reproducible.

In contrast, Friend et al., which primarily rely on a simplified cell model provided by yeast cells, find that:

> The methods of the present invention include: (i) obtaining or providing response profiles for the biological response (or responses) of interest; (ii) defining sets of co-regulated cellular constituents (i.e., genesets) in the response profiles; and (iii) identifying common response motifs among the defined sets of co-regulated cellular constituents which are associated with particular biological responses such as drug effectiveness or toxicity. The common response motifs thereby identified comprise the consensus profiles of the invention. In preferred embodiments, the methods of the invention further include the step (iv) of "projecting" the original response profiles onto the genesets identified in step (ii) above. Simplified, reduced-dimension response profiles are thereby produced which are more simply and robustly related to biological properties such as drug effectiveness and toxicity.

In contrast, Applicants' invention does not define sets of co-regulated cellular constituents (step ii above) or step (iii) above, defining common response motifs among the sets. Friend et al. require these steps because the reference is directed to deriving a consensus profile from a desired or "ideal" agent (sifting through all of the transcripts to find a set that reproducibly co-regulates). In the case of Friend *et al.*, for every "ideal" agent that they would like to compare against, they will derive a different consensus profile. Thus, every different consensus profile will contain a different gene set. In the present invention, the same cellular constituents (i.e. readout parameters) are determined for all test agents, and cellular constituents that are not altered by the "ideal" agent are just as important as those that are up or down-regulated. Indeed, Friend et al. emphasizes the use of co-varying cellular constituents, whereas the present invention selects cellular constituents that preferably do not co-vary, and are independent.

RELIEF REQUESTED

The Appellants respectfully request that the rejection of Claims 17 and 22 under 35 U.S.C. § 102(b) and the rejection of Claims 17 and 19-22 under 35 U.S.C. §103(a) be reversed; and that the application be remanded to the Examiner with instructions to issue a Notice of Allowance.

Respectfully submitted,

Date: August 10, 2009

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